

WHAT IS CLAIMED IS:

1. A mammalian cell comprising an isolated first strand of RNA of 15 to 30 nucleotides in length, and an isolated second strand of RNA of 15 to 30 nucleotides in length, wherein the first strand comprises a sequence that is complementary to at least 15 contiguous nucleotides of a targeted gene of interest, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a small interfering RNA (siRNA) duplex under physiological conditions, and wherein the siRNA silences only one allele of the targeted gene in the cell.
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2. The mammalian cell of claim 1, wherein the duplex is between 15 and 25 base pairs in length.
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3. The mammalian cell of any of the preceding claims, wherein the first and/or second strand further comprise an overhang region.
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4. The mammalian cell of any of the preceding claims, wherein the first and/or second strand further comprises a 3' overhang region, a 5' overhang region, or both 3' and 5' overhang regions.
5. The mammalian cell of claims 3 or 4, wherein the overhang region is from 1 to 10 nucleotides in length.
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6. The mammalian cell of any of the preceding claims, wherein the first strand and the second strand are operably linked by means of an RNA loop strand to form a hairpin structure comprising a duplex structure and a loop structure.
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7. The mammalian cell of claim 6, wherein the loop structure contains from 4 to 10 nucleotides.
8. The mammalian cell of claim 6, wherein the loop structure contains 4, 5 or 6 nucleotides.

9. A mammalian cell comprising an expression cassette encoding an isolated first strand of RNA of 15 to 30 nucleotides in length, and an isolated second strand of RNA of 15 to 30 nucleotides in length, wherein
5 the first strand comprises a sequence that is complementary to at least 15 contiguous nucleotides of a targeted gene of interest, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a small interfering RNA (siRNA) duplex under physiological conditions, and wherein the siRNA silences only one allele
10 of the targeted gene in the cell.
10. The mammalian cell of claim 9, wherein the expression cassette further comprises a promoter.
- 15 11. The mammalian cell of claim 10, wherein the promoter is a regulatable promoter.
12. The mammalian cell of claim 10, wherein the promoter is a constitutive promoter.
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13. The mammalian cell of claim 10, wherein the promoter is a CMV, RSV, pol II or pol III promoter.
- 25 14. The mammalian cell of claim 9, wherein the expression cassette further comprises a polyadenylation signal.
15. The mammalian cell of claim 14, wherein the polyadenylation signal is a synthetic minimal polyadenylation signal.
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16. The mammalian cell of claim 9, further comprising a marker gene.
17. The mammalian cell of any of claims 9-16, wherein the expression cassette is contained in a vector.

18. The mammalian cell of any of claims 9-17, wherein the vector is an adenoviral, lentiviral, adeno-associated viral (AAV), poliovirus, HSV, or murine Maloney-based viral vector.

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19. The mammalian cell of any of the preceding claims, wherein the vector is an adenoviral vector.

10 20. The mammalian cell of any of the preceding claims, wherein the targeted gene is a gene associated with a condition amenable to siRNA therapy.

21. The mammalian cell of any of the preceding claims, wherein alleles of the gene differ by seven or fewer base pairs out of 21 base pairs.

15 22. The mammalian cell of any of the preceding claims, wherein the gene is a beta-glucuronidase gene.

23. The mammalian cell of any of the preceding claims, wherein the alleles are murine-specific and human-specific alleles of beta-glucuronidase.

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24. The mammalian cell of any of the preceding claims, wherein alleles of the gene differ by one base pair out of 21 base pairs.

25 25. The mammalian cell of claim 24, wherein the gene encodes a transcript for TorsinA, Ataxin-3, Tau or huntingtin.

30 26. An isolated RNA duplex comprising a first strand of RNA and a second strand of RNA, wherein the first strand comprises at least 15 contiguous nucleotides complementary to mutant *TorsinA* transcript encoded by SEQ ID NO:55, and wherein the second strand is complementary to at least 12 contiguous nucleotides of the first strand.

27. The RNA duplex of claim 26, wherein the first strand of RNA is encoded by SEQ ID NO:49 (mutA-si).
28. The RNA duplex of claim 26, wherein the second strand of RNA is encoded by SEQ ID NO:50 (mutA-si).
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29. The RNA duplex of claim 26, wherein the first strand of RNA is encoded by SEQ ID NO:51 (mutB-si).
- 10 30. The RNA duplex of claim 26, wherein the second strand of RNA is encoded by SEQ ID NO:52 (mutB-si).
- 15 31. The RNA duplex of claim 26, wherein the first strand of RNA is encoded by SEQ ID NO:53 (mutC-si).
32. The RNA duplex of claim 26, wherein the second strand of RNA is encoded by SEQ ID NO:54 (mutC-si).
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33. An RNA duplex comprising a first strand of RNA and a second strand of RNA, wherein the first strand comprises at least 15 contiguous nucleotides complementary to mutant *Ataxin-3* transcript encoded by SEQ ID NO:8, and wherein the second strand is complementary to at least 12 contiguous nucleotides of the first strand.
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- 25 34. The RNA duplex of claim 33, wherein the first strand of RNA is encoded by SEQ ID NO:19 (siC7/8).
35. The RNA duplex of claim 33, wherein the second strand of RNA is encoded by SEQ ID NO: 20 (siC7/8).
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36. The RNA duplex of claim 33, wherein the first strand of RNA is encoded by SEQ ID NO:21 (siC10).

37. The RNA duplex of claim 33, wherein the second strand of RNA is encoded by SEQ ID NO:22 (siC10).
38. An RNA duplex comprising a first strand of RNA and a second strand of RNA, wherein the first strand comprises at least 15 contiguous nucleotides complementary to mutant Tau transcript encoded by SEQ ID NO:39 (siA9/C12), and wherein the second strand is complementary to at least 12 contiguous nucleotides of the first strand.
39. The RNA duplex of claim 38, wherein the second strand of RNA is encoded by SEQ ID NO:40 (siA9/C12).
40. The RNA duplex of any of claims 26-39, wherein the duplex is between 15 and 30 base pairs in length.
41. The RNA duplex of any of claims 26-40, wherein the duplex is between 19 and 25 base pairs in length.
42. The RNA duplex of any of claims 26-41, wherein the first and/or second strand further comprises an overhang region.
43. The RNA duplex of any of claims 26-42, wherein the first and/or second strand further comprises a 3' overhang region, a 5' overhang region, or both 3' and 5' overhang regions.
44. The RNA duplex of claim 42, wherein the overhang region is from 1 to 10 nucleotides in length.
45. The RNA duplex of any of claims 26-44, wherein the first strand and the second strand are operably linked by means of an RNA loop strand to form a hairpin structure comprising a duplex structure and a loop structure.

46. The RNA duplex of claim 45, wherein the loop structure contains from 4 to 10 nucleotides.
47. The RNA duplex of claim 45, wherein the loop structure contains 4, 5 or 6 nucleotides.
48. An expression cassette comprising a nucleic acid encoding at least one strand of the RNA duplex of any of claims 26-47.

10 49. The expression cassette of claim 48, further comprising a promoter.

50. The expression cassette of claim 49, wherein the promoter is a regulatable promoter.

15 51. The expression cassette of claim 49, wherein the promoter is a constitutive promoter.

52. The expression cassette of claim 49, wherein the promoter is a CMV, RSV, pol II or pol III promoter.

20 53. The expression cassette of any of claims 48-52, wherein the expression cassette further comprises a polyadenylation signal.

54. The expression cassette of claim 53, wherein the polyadenylation signal is a synthetic minimal polyadenylation signal.

25 55. The expression cassette of any of claims 48-54, further comprising a marker gene.

- 30 56. A vector comprising the expression cassette of any of claims 48-55.
57. A vector comprising two expression cassettes, a first expression cassette comprising a nucleic acid encoding the first strand of the RNA duplex of

any of claims 26-47 and a second expression cassette comprising a nucleic acid encoding the second strand of the RNA duplex of any of claims 26-47.

5 58. A cell comprising the expression cassette of any of claims 48-55.

10 59. The cell of claim 58, wherein the cell is a mammalian cell.

15 60. A non-human mammal comprising the expression cassette of any of claims 48-55.

20 61. A method of performing allele-specific gene silencing in a mammal comprising administering to the mammal an isolated first strand of RNA of 15 to 30 nucleotides in length, and an isolated second strand of RNA of 15 to 30 nucleotides in length, wherein the first strand comprises a sequence that is complementary to at least 15 contiguous nucleotides of a targeted gene of interest, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a small interfering RNA (siRNA) duplex under physiological conditions, and wherein the siRNA silences only one allele of the targeted gene in the mammal.

25 62. The method of claim 61, wherein alleles of the gene differ by seven or fewer base pairs out of 21 base pairs.

30 63. The method of claim 61, wherein the gene is a beta-glucuronidase gene.

64. The method of claim 61, wherein the alleles are murine-specific and human-specific alleles of beta-glucuronidase.

35 65. The method of claim 61, wherein alleles of the gene differ by one base pair out of 21 base pairs.

66. The method of claim 65, wherein the gene encodes a transcript for TorsinA, Ataxin-3, Tau or huntingtin .
67. The method of claim 61, wherein the targeted gene is a gene associated with a condition amenable to siRNA therapy.
68. The method of claim 67, wherein the condition amenable to siRNA therapy is a neurodegenerative disease.
- 10 69. The method of claim 68, wherein the neurodegenerative disease is a trinucleotide-repeat disease.
70. The method of claim 69, wherein the trinucleotide-repeat disease is a disease associated with polyglutamine repeats.
- 15 71. The method of claim 70, wherein the trinucleotide-repeat disease is Huntington's disease or a spinocerebellar ataxia (SCA).
72. The method of claim 71, wherein the SCA is SCA1, SCA2, SCA3, SCA6, SCA7, or SCA17.
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73. The method of claim 61, wherein the targeted gene encodes a ligand for a chemokine involved in the migration of a cancer cell, or a chemokine receptor.
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74. A method of substantially silencing a targeted allele while allowing substantially continued expression of a wild-type allele comprising contacting a cell with an expression cassette, wherein the expression cassette comprises a nucleic acid sequence encoding a small interfering RNA molecule (siRNA) targeted against the targeted allele, wherein expression from the targeted allele is substantially silenced but wherein expression of the wild-type allele is not substantially silenced.
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75. A method of treating dominantly inherited disease in an allele-specific manner comprising administering to a patient in need thereof an expression cassette, wherein the expression cassette comprises a nucleic acid sequence encoding a small interfering RNA molecule (siRNA) targeted against a targeted allele, wherein expression from the targeted allele is substantially silenced but wherein expression of the wild-type allele is not substantially silenced.

10 76. A method of performing allele-specific gene silencing comprising administering an expression cassette comprising a pol II promoter operably-linked to at least one strand of a nucleic acid encoding a small interfering RNA molecule (siRNA) targeted against a gene of interest, wherein the siRNA silences only one allele of a gene.

15 77. A method of performing allele-specific gene silencing in a mammal comprising administering to the mammal a vector comprising an expression cassette, wherein the expression cassette comprises a nucleic acid encoding at least one strand a small interfering RNA molecule (siRNA) targeted against a gene of interest, wherein the siRNA silences only one allele of a gene.

20 78. A method of screening of allele-specific siRNA duplexes comprising
(a) contacting a cell containing a predetermined mutant allele with an siRNA with a known sequence,
(b) contacting a cell containing a wild-type allele with an siRNA with a known sequence, and
(c) determining if the mutant allele is substantially silenced while the wild-type allele retains substantially normal activity.

25 79. A method of screening of allele-specific siRNA duplexes comprising
(a) contacting a cell containing a predetermined mutant allele and a wild-type allele with an siRNA with a known sequence, and

(b) determining if the mutant allele is substantially silenced while the wild-type allele retains substantially normal activity.

80. A method for determining the function of an allele comprising:

5 (a) contacting a cell containing a predetermined allele with an siRNA with a known sequence, and
(b) determining if the function of the allele is substantially modified.

81. A method for determining the function of an allele comprising:

10 (a) contacting a cell containing a predetermined mutant allele and a wild-type allele with an siRNA with a known sequence, and
(b) determining if the function of the allele is substantially modified while the wild-type allele retains substantially normal function.

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